

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

AMENDMENTS TO THE CLAIMS

1. (currently amended) A method for detecting reducing a test sensitivity to a beta-lactam family of antibiotics in a microbial growth inhibition test, the test capable of detecting both the beta-lactam family and at least the presence of an antibiotic in a test sample, whereby said antibiotic can be an antibiotic from a beta-lactam family, an antibiotic from a one additional family of antibiotics in a sample, multiple antibiotics all from the same family or multiple antibiotics from a combination of families, comprising the steps of:
 - a) combining the sample with both preparing a microbial culture and a microbial receptor, the culture comprising a microbial species susceptible to both the beta-lactam family and the at least one additional family of antibiotics, the microbial receptor characterized by its sensitivity to the beta-lactam family, the microbial receptor having been extracted from a bacteria with sensitivity to the beta-lactam family, whereby contacting the susceptible microbial species with an antibiotic to which the culture is susceptible either within said beta-lactam family or one additional family causes growth inhibition in the culture;
 - b) adding a microbial receptor with sensitivity to beta-lactams to the culture, the microbial receptor having been extracted from a bacteria, the bacteria having sensitivity to beta-lactams;
 - c) combining the sample and the culture;
 - d) growing the culture; and
 - e) determining an amount of culture growth,wherein a lack of culture growth reflects a presence of an antibiotic and wherein the test sensitivity to the beta-lactam family is reduced by adding combining the sample with the microbial receptor.

1. (currently amended) A method for detecting the presence of an antibiotic in a test sample, whereby said antibiotic can be an antibiotic from a beta-lactam family, an antibiotic from a one additional family, multiple antibiotics all from the same family or multiple antibiotics from a combination of families, comprising the steps of:
 - a) combining the sample with both a microbial culture and a microbial receptor, the culture comprising a microbial species susceptible to both the beta-lactam family and the one additional family of antibiotics, the microbial receptor characterized by its sensitivity to the beta-lactam family, the microbial receptor having been extracted from a bacteria with sensitivity to the beta-lactam family, whereby contacting the susceptible microbial species with an antibiotic to which the culture is susceptible causes growth inhibition in the culture;
 - b) growing the culture; and
 - c) determining an amount of culture growth,
wherein a lack of culture growth reflects a presence of an antibiotic and wherein the test sensitivity to the beta-lactam family is reduced by combining the sample with the microbial receptor.
2. (previously presented) The method of claim 1 further comprising extracting the antibiotic from the sample and adding an extract to the culture.
3. (previously presented) The method of claim 2 wherein the extracting comprises adding the sample to a solution comprising a Trizma Base.
4. (previously presented) The method of claim 2 wherein the extracting comprises adding the sample to a solution comprising a Potassium Phosphate.
5. (previously presented) The method of claim 2 wherein the extracting comprises adding the sample to a solution comprising a Potassium Phosphate Monobasic.
6. (previously presented) The method of claim 2 wherein the extracting comprises adding the sample to a solution of a pH between about 7.0 to about 8.0.

7. (previously presented) The method of claim 2 wherein the extracting comprises adding the sample to a solution of a pH of about 7.5.
8. (original) The method of claim 2 wherein the extracting comprises adding the sample to a solution comprising Potassium Phosphate Monobasic and Trizma Base at a pH of about 7.5.
9. (previously presented) The method of claim 1 wherein the microbial receptor is derived from the susceptible microbial species.
10. (previously presented) The method of claim 1 wherein the microbial receptor is added to the culture prior to adding the sample to the culture.
11. (canceled)
12. (original) The method of claim 1 wherein the antibiotic to be detected comprises a beta-lactam.
13. (canceled)
14. (previously presented) The method of claim 1 wherein the microbial receptor comprises a beta-lactam binding protein.
15. (previously presented) The method of claim 1 wherein the microbial receptor comprises an enzyme.
16. (previously presented) The method of claim 1 wherein the microbial receptor comprises a beta-lactam receptor extracted from an organism of a genus *Bacillus*.

17. (previously presented) The method of claim 1 wherein the microbial receptor comprises a beta-lactam receptor extracted from a *Bacillus stearothermophilus*.
18. – 23. (canceled)
24. (previously presented) The method of claim 1 wherein growing the culture comprises incubating at above a room temperature.
25. (original) The method of claim 1 wherein determining the amount of culture growth comprises observing a color change within the culture.
26. (original) The method of claim 1 further comprising a colorimetric assay in which a color change reflects a change in the pH of the culture.
27. (previously presented) The method of claim 26 wherein a reagent for the colorimetric assay is combined within the microbial culture.
28. (previously presented) The method of claim 1 wherein the microbial culture comprises a bacterial spore.
29. (previously presented) The method of claim 1 wherein the microbial culture comprise spores of *Bacillus stearothermophilus*.
30. (previously presented) The method of claim 1 further comprising the step of adding to the microbial culture at least two buffers, wherein a first buffer has a pKa of above about 7 and a second buffer has a pKa of below about 7.
31. (original) The method of claim 30 wherein one of said at least two buffers has a pKa of about 8 to about 11 and the other of said buffers has a pKa of about 4.5 to about 6.5.

32. (previously presented) The method of claim 30 wherein one of said buffers comprises a succinate.

33. (previously presented) The method of claim 30 wherein one of said buffers comprises a borate.

34. (previously presented) The method of claim 30 wherein one of said buffers comprises a Trizma Base.

35. (previously presented) The method of claim 1 wherein a pH of the microbial culture, prior to adding the sample, is above about a pH of 7.5.

36. (previously presented) The method of claim 1 wherein a pH of the microbial culture, prior to adding the sample, is above about a pH of 8.0.

37. (previously presented) The method of claim 1 wherein a pH of the microbial culture, prior to adding the sample, is between about a pH of 8.0 and about a pH of 10.5.

38. (withdrawn) A method for detection of an antibiotic in a sample comprising:

a) preparing a microbial culture that will have growth inhibition when contacted with an antibiotic;

b) buffering the culture with at least two buffers, wherein one of the buffers has a pKa of above 7 and one of the buffers has a pKa of below 7;

b) adding the sample to the culture;

c) growing the culture;

d) determining the amount of culture growth,

wherein lack of culture growth indicates the presence in the sample of a microbial growth inhibitor.

39. (withdrawn) The method of claim 38 wherein the buffers are added to the culture prior to contacting the culture with the sample.

40. (withdrawn) The method of claim 38 wherein at least one of the two buffers is added to the culture concurrently with the sample.

41. (withdrawn) The method of claim 38 wherein at least one of the two buffers is added to the culture subsequent to adding the sample.

42. (withdrawn) The method of claim 38 wherein one of said at least two buffers has a pKa of about 8 to about 11 and the other of said buffers has a pKa of about 4.5 to about 6.5.

43. (withdrawn) The method of claim 38 wherein one of said buffers is succinate.

44. (withdrawn) The method of claim 38 wherein one of said buffers is borate.

45. (withdrawn) The method of claim 38 wherein one of said buffers is Trizma Base.

46. (withdrawn) The method of claim 38 further comprising a method for reducing growth inhibition of an antibiotic comprising contacting the culture with a substance that reduces the culture growth inhibition of the antibiotic.

47. (withdrawn) The method of claim 38 wherein the pH of the culture, prior to adding the sample, is between about pH 7.5 and about pH 10.5.

48. (withdrawn) The method of claim 38 wherein the culture comprises spores of *Bacillus stearothermophilus*.

49. (withdrawn) The method of claim 46 wherein the inhibition reducing substance comprises a protein isolated from *Bacillus stearothermophilus*.

50. (withdrawn) The method of claim 38 wherein the culture comprises spores of *Bacillus stearothermophilus* and wherein the inhibition reducing substance comprises a protein isolated from *Bacillus stearothermophilus*.

51. (withdrawn) The method of claim 50 wherein the culture is sensitive to beta-lactams and the inhibition reducing substance comprises a beta-lactam binding protein isolated from an organism from the genus *Bacillus*.

51. (withdrawn) A method for the microbial culture, growth inhibition, detection of microbial growth inhibitors in a test sample, at reduced sensitivity levels for one or more, but not all, antibiotics to which a culture is sensitive, said method comprising:

- a) adding a sample to the culture, the culture comprising spores of *Bacillus stearothermophilus*, agar, at least two buffers, one of said at least two buffers having a pKa of above 7 and the other of said buffers having a pKa of below 7, a substance that reduces the microbial growth inhibition of an antibiotic, and a pH indicators;
- b) incubating the culture with the sample;
- c) detecting a change in pH,

wherein a pH below 7 reflects culture growth and wherein culture growth reflects the absence of inhibitors in the sample at above a preset threshold level for certain antibiotics.

52. (withdrawn) The method of claim 51 wherein the pH of the culture, prior to sample addition, is about pH 8.

53. (withdrawn) A method for detection of an antibiotic in a sample comprising:

- a) preparing a microbial culture that will have growth inhibition when contacted with an antibiotic, the culture, prior to use, characterized in that the pH is above about pH 8.0;
- b) adding the sample to the culture;
- c) growing the culture;
- d) determining the amount of culture growth,

wherein lack of culture growth indicates the presence in the sample of a microbial growth inhibitor.

54. (withdrawn) The method of claim 53 wherein the pH of the culture is between about pH 8.0 and about pH 10.5.

55. (withdrawn) The method of claim 53 further comprising a method for reducing growth inhibition of a selected antibiotic to which the culture is sensitive comprising contacting the culture with a substance that reduces the culture growth inhibition of the antibiotic.

56. (withdrawn) The method of claim 53 wherein the culture comprises: pH indicator, glucose, Mueller-Hinton broth, agar and spores.

57. (withdrawn) The method of claim 53 wherein the culture comprises spores of *Bacillus stearothermophilus*.

58. (withdrawn) The method of claim 55 wherein the substance that reduces the culture growth inhibition of the antibiotic is derived from a microbe of the genus *Bacillus*.

59. (withdrawn) A test apparatus for the microbial culture, growth inhibition, detection of microbial growth inhibitors in a test sample, at reduced sensitivity levels for certain antibiotics, said test apparatus comprising:

- a) a vial containing a microbial culture, the culture comprising: (i) agar, (ii) spores, (iii) nutrients, (iv) a protein that binds an antibiotic, (v) pH indicator;
- b) means to transfer into the vial a sample to be tested for antibiotics, wherein the test sample is added to the culture and the culture is allowed to grow, and wherein the growth of the culture, or lack thereof, indicates the presence in the sample of microbial growth inhibitors.

60. (withdrawn) The test apparatus of claim 59 further comprising a an extraction reagent within a container, the container located within the vial and above the culture, the container comprising: (i) a cylinder having a one open end and an other opposite open end, (ii) a probe-puncturable membrane seal over the open ends to form a sealed compartment, (iii) and an extraction reagent sealed within the sealed compartment.

61. (withdrawn) The test apparatus of claim 59 further comprising within the culture at least two buffers wherein one of said buffers has a pKa of above 7 and the other of said buffers has a pKa of below 7.

62. (withdrawn) The test apparatus of claim 59 wherein the spores are spores of *Bacillus stearothermophilus*.

63. (withdrawn) The test apparatus of claim 59 further characterized in that the antibiotic binding protein comprises a protein isolated from *Bacillus stearothermophilus*.

64. (withdrawn) The test apparatus of claim 59 further characterized in that the antibiotic binding protein comprises an antibody.

65. (withdrawn) The apparatus of claim 60 wherein the extraction reagent comprises a mixture of Trizma Base and Potassium Phosphate Monobasic.

66. (withdrawn) The apparatus of claim 61 wherein one of said buffers is Trizma Base and the other of said buffers is succinate.

67. (new) A method for preparing a microbial growth inhibition test, the test capable of detecting the presence of an antibiotic in a test sample, whereby said antibiotic can be an antibiotic from a beta-lactam family, an antibiotic from a one additional family, multiple antibiotics all from the same family or multiple antibiotics from a combination of families, comprising the steps of:

a) preparing a microbial culture, the culture comprising a microbial species susceptible to both the beta-lactam family and the at least one additional family of antibiotics, whereby contacting the susceptible microbial species with an antibiotic to which the microbial species is susceptible causes growth inhibition in the culture;

b) adding a microbial receptor with sensitivity to beta-lactams to the culture, the microbial receptor from a bacteria, the bacteria having sensitivity to beta-lactams, wherein when the sample and the culture are combined the culture can grow and wherein a lack of culture growth reflects a presence of one or multiple antibiotics and wherein the test sensitivity to the beta-lactam family is reduced by adding the microbial receptor.

68. (new) The method of claim 67 wherein the microbial receptor is derived from the susceptible microbial species.

69. (new) The method of claim 67 wherein the microbial receptor is added to the culture prior to adding the sample to the culture.

70. (new) The method of claim 67 wherein the antibiotic to be detected comprises a beta-lactam.

71. (new) The method of claim 67 wherein the microbial receptor comprises a beta-lactam binding protein.

72. (new) The method of claim 67 wherein the microbial receptor comprises an enzyme.

73. (new) The method of claim 67 wherein the microbial receptor comprises a beta-lactam receptor isolated from an organism of a genus *Bacillus*.

74. (new) The method of claim 67 wherein the microbial receptor comprises a beta-lactam receptor extracted from a *Bacillus stearothermophilus*.

75. (new) The method of claim 67 wherein the microbial culture comprises a bacterial spore.

76. (new) The method of claim 67 wherein the microbial culture comprise spores of *Bacillus stearothermophilus*.

77. (new) The method of claim 67 wherein a pH of the microbial culture, prior to adding the sample, is above about a pH of 7.5.

78. (new) The method of claim 67 wherein a pH of the microbial culture, prior to adding the sample, is above about a pH of 8.0.

79. (new) The method of claim 67 wherein a pH of the microbial culture, prior to adding the sample, is between about a pH of 8.0 and about a pH of 10.5.